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ABSTRACT

Holstein cows fed concentrate:hay diets also were fed for 14 days supplements of soybean oil plus casein, soybean oil protected from ruminal hydrogenation by encapsulation in a casein-formaldehyde matrix, cottonseed oil plus casein, or cottonseed oil protected with casein-formaldehyde. The supplements were fed at rates to give a linoleic acid (18:2) intake of 225 g/day. Yields of milk and milk protein were not affected by treatment. Milk 18:2 was not increased by the unprotected soybean oil or cottonseed oil but was increased by protected soybean and cottonseed oil from a control of 2.3 to 5.7% of total milk fat. Milk 18:0 and 18:1 also increased. Compensatory declines were observed in milk 16:0 and 14:0 acids. In fecal fatty acids during the treatment periods, percentage of 18:2 of the total fat decreased and 18:0 markedly increased. These results indicate hydrogenation of the dietary oils in the alimentary tract or a differential absorption. Fecal 16:0 and 14:0 decreased.

INTRODUCTION

The fatty acid composition of milk fat can be modified to a higher degree of unsaturation than usual in milk fat by feeding diets containing polyunsaturated vegetable oils protected from ruminal hydrogenation by encapsulation in a protein-formaldehyde coat (2, 5, 16, 17, 18). At Beltsville the use of protected safflower oil has been studied extensively, both for brief treatment (1, 8) and for complete lactations (27). When protected safflower oil was fed, the linoleic acid concentration of milk fat and the cholesterol of plasma were increased; and when vitamin E was fed, the oxidized flavor in milk was reduced (8). The objective of the present experiment was to determine changes in milk fat composition after feeding relatively small amounts of two widely available vegetable oils, soybean oil or cottonseed oil, which were encapsulated in a casein-formaldehyde coating.

EXPERIMENTAL PROCEDURES

Eight Holstein cows in at least the 5th mo of lactation were fed in a sequential experiment with a 2-wk period sequence of control, soybean oil, control, soybean oil, control, cottonseed oil, and control for a total of seven periods. During control periods all eight cows were fed concentrate and hay. During oil feeding periods the eight cows were assigned randomly to a supplement of either unprotected oil plus casein or oil plus casein treated with formaldehyde to give a linoleic acid (18:2) intake from supplement of approximately 225 g/day per cow. The hay and concentrate were fed at 1:1 ratio on air dry weight basis and offered to the animal on estimated requirements for digestible energy (14). The requirement for digestible energy for each cow was calculated and adjusted at the end of each treatment period. The ground hay (5.1-cm screen) consisted of medium quality alfalfa-orchardgrass (about 60:40) mixture. Composition of the hay and concentrates is in Table 1. Cell wall was determined by the procedure described by Goering and Van Soest (7). During treatment, the diet contained about 5.8% lipid; the control hay:concentrate diet contained 3.3% lipid. Oil:casein and oil:casein:formaldehyde particles were prepared by homogenization and spray drying as described by Plowman et al. (17). The composition of the particles

TABLE 1. Composition determined chemically of the hay[a] and the five concentrate[b] mixes.

Treatment	Hay	Concen-trate	Oil plus concentrate: Soybean, Formaldehyde −	Soybean, Formaldehyde +	Cottonseed, Formaldehyde −	Cottonseed, Formaldehyde +
Crude protein (% DM)	17.1	16.6	18.1	18.0	18.2	18.1
Cell wall (% DM)	48	20	20	20	18	17
Lipid (% DM)	3.4	3.3	8.3	7.8	8.7	8.3
16:0 (% total lipid)	24.4	14.8	12.0	13.7	18.6	19.4
18:0 (% total lipid)	5.5	2.2	3.3	4.4	3.2	2.8
18:1 (% total lipid)	6.5	23.2	20.2	24.9	21.7	21.8
18:2 (% total lipid)	21.1	53.8	48.6	51.1	51.2	52.6

[a]A medium quality orchardgrass:alfalfa (about 60:40 ratio).

[b]Ingredients of the basal concentrate were: corn meal, 35.7%; barley, 4.6%; wheat middlings, 22.1%; corn gluten, 17.9%; oats, 4.3%; SBOM, (44%), 4.7%; molasses, 8.9%; trace mineralized salt, .9%; and dicalcium phosphate, .9%.

varied but was approximately 2/3 oil and 1/3 sodium caseinate with .5% water and .5% formaldehyde (100% basis). Composition of the oils is in Table 2. Mean intake and production values were from the last 7 days of each period.

Milk was sampled at each milking, and samples from each four consecutive milkings were composited for determination of percentage of fat (Foss Milko-Tester), protein (Kjeldahl), and solids-not-fat (SNF) by the Watson lactometer method. Samples from each two consecutive milkings were composited for determination of fatty acid composition of the milk fat as described previously (1).

The concentrate part of the diet contained Cr_2O_3 as an external marker for estimating lipid digestibility. On the 13th day of each 2 wk, total feces were collected except during the 4 h cows were going through the milking parlor. Feces were analyzed for fat (methanol:chloroform extraction) and fatty acid composition (1). The Cr_2O_3 concentration was determined on feces and feed (25) for calculating digestibility of the lipid by the ratio technique.

Blood samples were taken on days 7 and 14 of each period 4 h after feeding. Cholesterol, triglycerides, and nonesterified fatty acids in plasma were determined (8, 24). Body weights were taken in each 2-wk period.

Statistical comparisons were by analysis of variance between differences of treatments to pre- and post-treatment period means. The difference between control and treatment was determined by testing the treatment difference against zero (4).

TABLE 2. Fatty acid composition of vegetable oils in the experiments[a].

Fatty acid	Soybean	Cotton-seed
	Weight %	
16:0	10.9	21.0
18:0	3.7	2.8
18:1	22.1	19.3
18:2	54.9	54.4
18:3	7.0	.7
Others	1.4	1.8

[a]Chemically determined on cottonseed and soybean oil used in producing the protected and unprotected oil:casein particles.

RESULTS AND DISCUSSION

Feeding Soybean and Cottonseed Oil to Lactating Cows

Performance data are in Table 3. Mean daily intake of dry matter and intake as percentage of body weight were similar for control and for all treatments. Estimated digestible energy intake was several Mcal greater during treatment periods ($P<.05$) because of the oil supplement of approximately 4 Mcal/cow per day. Fat intake was higher ($P<.01$) during treatment by

TABLE 3. Daily feed and energy intake and production of milk and milk components of cows before and after feeding protected or unprotected vegetable oils.

	Soybean oil[a]				Cottonseed oil[b]			
		Formaldehyde				Formaldehyde		
Criterion	Control	–	+	SE[c]	Control	–	+	SE[c]
DM intake (kg/day)	14.2	14.8	14.1	.27	11.7	12.4	13.6	.63
DM intake (% BW)	2.18	2.27	2.17	.043	1.79	1.90	2.06	.101
DE intake (Mcal/day)	42.4[D]	46.9	44.9	.99	35.1[d]	40.1	43.9	2.00
Fat intake (g/day)	459[D]	858	805	18.2	392[D]	744	790	31.0
Milk (kg/day)	14.5	14.8	15.0	.37	9.5	9.8	11.6	.76
FCM (kg/day)	14.3[D]	14.6	15.8	.40	9.5	9.4	11.9	.89
Fat (%)	3.9[D]	3.9[e]	4.3	.11	4.0	3.9	4.2	.15
Fat (g/day)	567[D]	577[e]	644	15.8	382	367	487	39.6
Protein (%)	3.4	3.4	3.4	.02	3.6	3.7	3.5	.06
SNF (%)	8.7[D]	8.8[e]	8.9	.02	8.7	8.8	8.8	.05

[a]The control mean includes 24 individual observations and the treatment means 8 individual observations.

[b]The control mean includes 16 individual observations and the treatment means 4 individual observations.

[c]Standard error of a mean applies to treatment (no formaldehyde and formaldehyde) means.

[D]Control vs. treatments, P<.01.

[d]Control vs. treatments, P<.05.

[e]No formaldehyde vs. formaldehyde-treated oil:casein mixture, P<.05.

about 370 g or from 3.3% fat to about 5.8% of the total ration. Milk yield was not changed significantly by the addition of protected or unprotected oil in the diets. Fat-corrected milk production increased (P<.01) during soybean oil:casein supplementation. Fat percentage, fat yield, and percentage of SNF increased (P<.01) during feeding of soybean oil supplements. The protected soybean oil treatment produced a 4.3% fat concentration in milk; unprotected soybean oil produced a 3.9% concentration (P<.05). Solids-not-fat concentration was higher (P<.05) for milk from cows supplemented with protected soybean oil than from cows supplemented with the unprotected soybean oil. These findings suggest an increase in ash or lactose because protein did not increase. An increase in lactose with the feeding of soybean oil has been observed (11, 21). Fat-corrected milk, fat percentage and yield, protein percentage, and SNF were not different between treatments or between treatments and control periods for the cottonseed oil treatments. This may be due to the lower milk production during cottonseed oil supplementation; it was only about 70% of that of the soybean oil feeding periods.

Although this small addition of soybean or cottonseed oil did not affect milk, milk fat, or protein yields consistently, milk-fat composition was affected. Feeding soybean oil generally has produced decreased milk-fat percentage (10, 11, 13, 20, 21), but no explanation has been given for this decrease. Whole soybeans (10, 21) or soybean oil plus soybean meal (10) have prevented the decrease in milk-fat percentage. The unprotected oils fed in this experiment were coated with casein, and this may be related to lack of depression of milk-fat percentage. The effect of added oil may be on production of rumen volatile fatty acid, but VFA have not been measured. In a preliminary experiment, abomasal infusion of unsaturated oils to bypass the rumen increased milk-fat percentages. This agrees with the infusion work of Storry et al. (23). In contrast the decrease in milk-fat percentage when unprotected unsaturated oils are fed, suggests that the effect is in the rumen.

Figure 1 shows the changes in 18:2 in milk fat when the unprotected or protected soybean and cottonseed oils were fed. Addition of the unprotected oils to the diet caused no increase

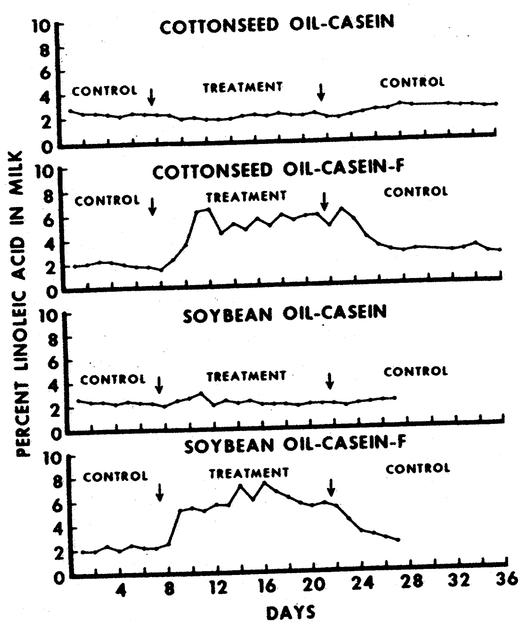

FIG. 1. Daily linoleic acid concentration in milk fat of cows fed protected and unprotected oil. The arrows indicate start and end of feeding the oil.

in milk-fat 18:2, but the casein-formaldehyde encapsulation caused a threefold increase (P<.01) to about 6% of total fatty acids.

Table 4 shows the changes in fatty acids in milk. Stearic (18:0) and oleic (18:1) acids were increased (P<.01) during treatment. Oleic acid was highest (P<.01) during the supplementation by unprotected oil:casein; this result indicates hydrogenation of one of the double bonds of the ingested 18:2. Palmitic acid (16:0) was lower (P<.01) during the soybean oil treatment; this result indicates a decline by dilution as greater quantities of C18 acids were transferred into milk.

Table 5 shows the efficiency of transfer of soybean and cottonseed oil 18:2 into milk fat. Because the C18 acids in milk originate almost entirely from circulating blood lipids, they serve as excellent markers for the overall efficiency of the transfer process. The high recoveries of 18:0 and 18:1 in Table 5 indicate hydrogenation of 18:2 and 18:3, which gives increased 18:0 and 18:1 acids. When protected soybean oil was fed, the 19% transfer for 18:2 agreed with previous experiments in which spray-dried casein-formaldehyde encapsulated

TABLE 4. Milk fat fatty acid composition during control and periods of feeding unprotected and protected soybean and cottonseed oils.

Fatty acid	Soybean oil				Cottonseed oil			
		Formaldehyde				Formaldehyde		
	Control	−	+	SE[a]	Control	−	+	SE[a]
	(weight %)							
4:0	5.6	4.5	4.8	.38	3.1[b]	2.8	2.4	.60
6:0	3.9[B]	3.1	3.2	.34	2.2[B]	1.7	1.4	.21
8:0	2.0[B]	1.5	1.6	.14	1.3[B]	.9	.8	.04
10:0	3.9[B]	2.8	2.9	.19	2.9[B]	1.9	1.8	.18
12:0	4.0[B]	2.9	3.0	.15	3.4[B]	2.2	2.5	.19
14:0	11.6[B]	9.3	8.9	.19	10.7[B]	7.9	9.0	.39
16:0	26.7[B]	23.1	20.7	1.00	26.0	24.0	25.9	1.12
18:0	9.1[B]	11.8	12.9	.61	11.5[B]	12.5[C]	15.0	.19
18:1	22.9[B]	32.2[C]	28.7	.63	27.4[B]	35.2[C]	30.0	.52
18:2	2.2[B]	2.0[C]	6.1	.28	2.4[B]	2.1[C]	5.3	.19
18:3	1.1[B]	1.8	1.9	.11	1.3[b]	2.1	1.4	.18
Others	6.7[B]	5.6	5.0	.16	7.5[B]	6.3[C]	5.1	.14

[a]Standard error of a mean applies to treatment (no formaldehyde and formaldehyde) means.

[B]Control vs. treatments, $P<.01$.

[b]Control vs. treatments, $P<.05$.

[C]No formaldehyde vs. formaldehyde-treated oil:casein mixture, $P<.01$.

vegetable oils were used (1, 8). The 11% transfer of 18:2 when protected cottonseed oil was fed is lower and suggests that either the oil was not as well protected as the soybean oil or that the 18:2 from the cottonseed oil was transferred less efficiently. The appearance of larger than expected amounts of 18:0 and 18:1 for all oil supplementations suggests incomplete protection.

Williams et al. (26) and Hutjens and Schultz (9) reported that feeding cracked, ground, or whole soybeans increased content of 18:2 in milk fat slightly. Steele et al. (22) found that feeding coarsely ground soybeans transferred about 24% of added 18:2, but feeding soybean oil transferred only 5% of added 18:2. Other workers (9, 15) have reported only slight increases in milk fat 18:2 when formaldehyde-treated full-fat soybean meal was fed. In contrast, Bitman et al. (2) and Mattos and Palmquist (12) fed formaldehyde-treated and untreated full-fat soybean flour preparations to lactating cows and found that milk yield, fat percentage, and 18:2 content were increased significantly by the experimental diets. Bitman et al. (2) found that the efficiency of transfer of added 18:2 was about 37%; this percentage indicates protection of the unsaturated lipid.

There have been only a few studies of the effects of feeding cottonseed oil upon composition of milk fat. Brown et al. (3) found that 6% added cottonseed oil decreased C6 through C14 acids in the milk fat and increased C18 acids. Steele and Moore (19) incorporated 10% cottonseed oil into the diet and found an increased percentage of 18:0 and 18:1 acids in the milk fat and a decreased percentage of 10:0, 12:0, 14:0, and 16:0 in the milk fat. In other studies (20), 5 to 20% cottonseed oil was added to experimental diets, and fatty acid changes were similar. Generally the inclusion of unprotected cottonseed oil increased the yield of C18 polyunsaturated fatty acids in the milk fat only slightly or not at all.

Table 6 shows fecal fatty acids. Fecal 18:2 decreased ($P<.01$) when either unprotected or protected soybean or cottonseed oil was fed. Fecal 18:1 did not change, but fecal 18:0 increased ($P<.05$), and fecal 16:0 decreased ($P<.05$). A comparison of the changes of fecal fatty acids induced by soybean oil or soybean oil-formaldehyde feeding with changes induced by cottonseed oil or cottonseed oil-formaldehyde feeding indicated several significant differ-

TABLE 5. Daily transfer into milk fat of 18 fatty acids fed[a].

Fatty acid	Intake			Excess in milk			Recovery[b]	
	Formaldehyde			Formaldehyde			Formaldehyde	
	–	+	SE[c]	–	+	SE[c]	–	+
	(g)			(g)			(%)	
				Soybean oil				
18:0	15	20	1.9	15	32	3.8	100	160
18:1	65	79	9.9	47	57	6.9	72	72
18:2	169	155	13.4	-1	30	3.3	-10[D]	19
Total	249	254	25.0	61	119	12.2	24	47
				Cottonseed oil				
18:0	6	6	2.2	1	29	7.5	17	483
18:1	74	81	8.0	23	43	16.8	31	53
18:2	172	199	19.7	-1	21	5.7	-1[d]	11
Total	252	286	29.6	24	92	29.0	10	32

[a]Calculated excess intake and yield of treatments compared with control feeding and yield.

[b]Recovery was calculated by using the means of the excess intake and excess in milk. Individual values were used to test significance; only 18:2 was significantly different.

[c]Standard error of a mean applies to treatment (no formaldehyde and formaldehyde) means.

[D]No formaldehyde vs. formaldehyde-treated oil:casein mixture, $P<.01$.

[d]No formaldehyde vs. formaldehyde-treated oil:casein mixture, $P<.05$.

TABLE 6. Fatty acid concentrations of feces from cows fed unprotected and protected soybean and cottonseed oils.

	Soybean oil				Cottonseed oil			
		Formaldehyde				Formaldehyde		
Constituent	Control	–	+	SE[a]	Control	–	+	SE[a]
Fecal lipid (g/day)	255	289	224	. . .	207	210	256	. . .
Fat (% DM)	4.8[B]	5.8	5.3	.29	5.2	5.3	5.1	.03
Fatty acids (weight %)								
14:0	3.4[B]	1.9	1.7	.43	4.3	4.2	4.6	.16
16:0	22.3[B]	14.7	16.8	1.13	22.8[b]	18.8[c]	21.3	.72
18:0	40.6[B]	59.5	60.7	3.72	37.0[b]	48.0[c]	44.3	1.30
18:1	11.4	11.6[c]	9.3	.54	11.0	11.2	12.0	1.13
18:2	6.2[B]	3.3	2.5	.64	5.3[B]	3.6[c]	2.8	.83
Others	16.1[B]	8.9	8.9	1.74	19.6[b]	14.2[c]	14.9	1.33

[a]Standard error of a mean applies to treatment (no formaldehyde and formaldehyde) means.

[B]Control vs. treatments, $P<.01$.

[b]Control vs. treatments, $P<.05$.

[c]No formaldehyde vs. formaldehyde-treated oil:casein mixture, $P<.05$.

TABLE 7. Blood plasma lipids and milk cholesterol during the feeding of unprotected and protected soybean oil and cottonseed oil.

Constituent	Soybean oil				Cottonseed oil			
		Formaldehyde				Formaldehyde		
	Control	−	+	SE[a]	Control	−	+	SE[a]
Plasma cholesterol (mg/100 ml)	144B	153	167	6.0	134B	163	170	4.5
Plasma triglycerides (mg/100 ml)	11B	17	17	1.7	15	17	16	1.8
Nonesterified fatty acids (mg/100 ml)	4.2	4.0C	4.8	.16	4.9B	7.1	7.5	.14
Milk cholesterol (mg/100 ml)	14	14	13	.9	17	16	15	1.4

[a]Standard error of a mean applies to treatment (no formaldehyde and formaldehyde) means.

[B]Control vs. treatment, $P<.01$.

[C]No formaldehyde vs. formaldehyde-treated oil:casein mixture, $P<.01$.

ences (Table 6). Feces had much greater concentrations of 18:0 when soybean oil was fed than when cottonseed oil was fed; consequently, concentrations of 16:0 and 14:0 decreased when soybean oil was fed. Feeding of the oils increased fecal lipid. These increases were statistically significant ($P<.01$) when soybean oil was fed.

The changes in the fatty acid composition of the feces indicated hydrogenation of the unsaturated dietary oils in the alimentary tract. The slight increase in percentage of fecal lipid and the increase in the percentage of 18:0 in fecal lipid, together with the fact that only 10 to 20% of the dietary 18:2 was transferred into milk, may account for the disappearance of the 18:2 in the ingested vegetable oils. Dawson and Kemp (6), in studies with sheep, found a steadily increasing concentration of 18:0 in lipids of ingesta sampled further down the alimentary tract. They suggested that in addition to the rumen, there is another area of bihydrogenation in the lower digestive tract. Total fecal lipid produced daily per cow was calculated; total fecal excretions determined by Cr_2O_3 marker were used. There were no apparent differences in total fecal lipid when animals were fed protected and unprotected oil-supplemented diets. This similarly indicates that digestibility of lipid was similar for treatment diets, and additional lipid fed was digested almost completely. The proportion of fatty acid in feces indicates the actual yield of the individual fatty acids.

Changes in plasma cholesterol, triglycerides, and nonesterified fatty acids are in Table 7. Because the amount of additional fat fed daily was small, the increase in percentage of concentration of dietary lipids into blood was slight. During cottonseed oil feeding, whether protected or unprotected, plasma cholesterol and nonesterified fatty acids increased ($P<.01$). During soybean oil feeding, plasma triglycerides and cholesterol of both protected and unprotected fatty acids increased ($P<.01$). Plasma nonesterified fatty acids were elevated in cows receiving protected soybean oil as compared to cows receiving unprotected soybean oil. Milk cholesterol (Table 7) remained essentially constant during the periods when additional fat was fed.

Feeding of protected soybean oil and cottonseed oil at similar rates increased 18:2 in the milk fat. Milk fat percentage was increased by protection of soybean oil but not significantly by protection of cottonseed oil.

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